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United States Patent [19]

Sogabe et al.

[11] **Patent Number:** **6,080,553**[45] **Date of Patent:** ***Jun. 27, 2000**[54] **CREATINE AMIDINOHYDROLASE,
PRODUCTION THEREOF AND USE
THEREOF**3,907,644 9/1975 Mollering et al. 195/99
5,451,520 9/1995 Furukawa et al. 435/227[75] **Inventors:** Atsushi Sogabe; Takashi Hattori;
Yoshiaki Nishiya; Yoshihisa
Kawamura, all of Isuruga, Japan[73] **Assignee:** Toyo Boseki Kabushiki Kaisha,
Osaka, Japan[*] **Notice:** This patent issued on a continued prosecution application filed under 37 CFR 1.53(d), and is subject to the twenty year patent term provisions of 35 U.S.C. 154(a)(2).[21] **Appl. No.:** 08/799,897[22] **Filed:** Feb. 13, 1997[30] **Foreign Application Priority Data**

Feb. 13, 1996 [JP] Japan 8-025435

[51] **Int. Cl.⁷** C12Q 1/34; C12N 9/78;
C12N 1/20; C12N 1/00[52] **U.S. Cl.** 435/18; 435/227; 435/192;
435/252.3; 435/320.1; 435/829; 435/252.33[58] **Field of Search** 435/18, 227, 252.1,
435/320.1, 829, 192, 252.3, 252.33[56] **References Cited****U.S. PATENT DOCUMENTS**

3,806,420 4/1974 Holz et al. 195/66

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07 265074 10/1995 Japan .*Primary Examiner*—Elizabeth Slobodyansky
Attorney, Agent, or Firm—Leydig, Voit & Mayer, Ltd.[57] **ABSTRACT**

A creatine amidinohydrolase having the following physico-chemical properties:

Action: catalyzing the following reaction;
creatine+H₂O→sarcosine+urea

Optimum temperature: about 40–50° C.

Optimum pH: pH about 8.0–9.0

Heat stability: not more than about 50° C. (pH 7.5, 30 min)

Km value for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: about 3.5–10.0 mM

Molecular weight: about 43,000 (SDS-PAGE)

Isoelectric point: about 3.5,

a method for producing said enzyme, comprising culture of microorganism producing said enzyme, a method for the determination of creatine or creatinine in a sample using said enzyme, and a reagent therefor.

23 Claims, 2 Drawing Sheets

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